

Supporting Information for

Short Communication

**Berberine improves central memory formation of CD8⁺ T cells:
Implications for design of natural product-based vaccines**

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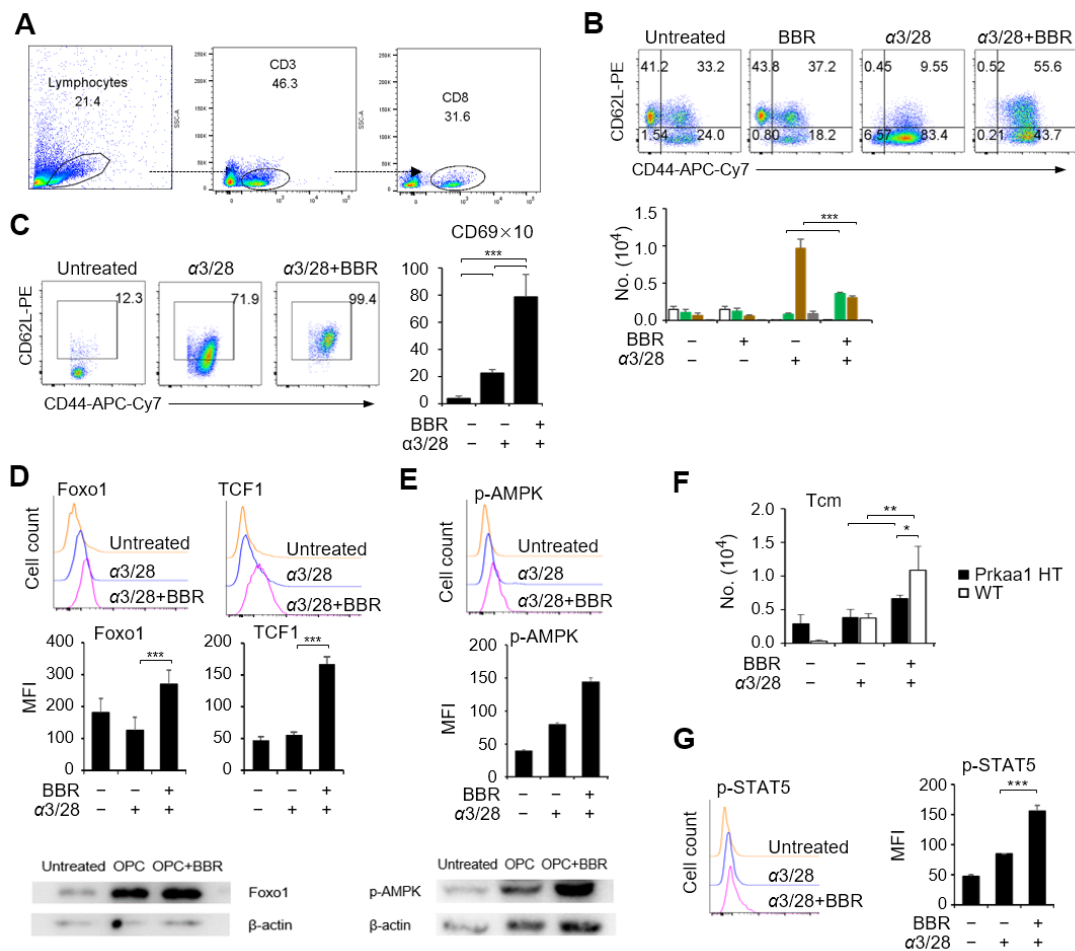


Figure S1 BBR upregulates Foxo1 and TCF1 through AMPK and Stat5 signaling. Splenic lymphocytes isolated from naïve mice were stimulated with α 3/28 in combination of BBR for 3 days. (A) Gating strategy of flow cytometry. (B) Flow cytometry analysis of CD62L⁺CD44⁺ Tcm and CD62L⁻CD44⁺ Tem/Teff CD8⁺ T cells in BBR-pretreated mice. CD62L⁺CD44⁻ naïve and CD62L⁻CD44⁻ double negative populations were also included. (C) Percentage of CD69⁺ cells in CD8⁺ T cells. Expression of Foxo1, TCF1 (D), p-AMPK (E), number of CD62L⁺CD44⁺ Tcm (F), and expression of p-Stat5 (G) in CD8⁺ T cells. Results are representative of two to five independent experiments ($n = 3-4$ per group). Data are shown as mean \pm SD; * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

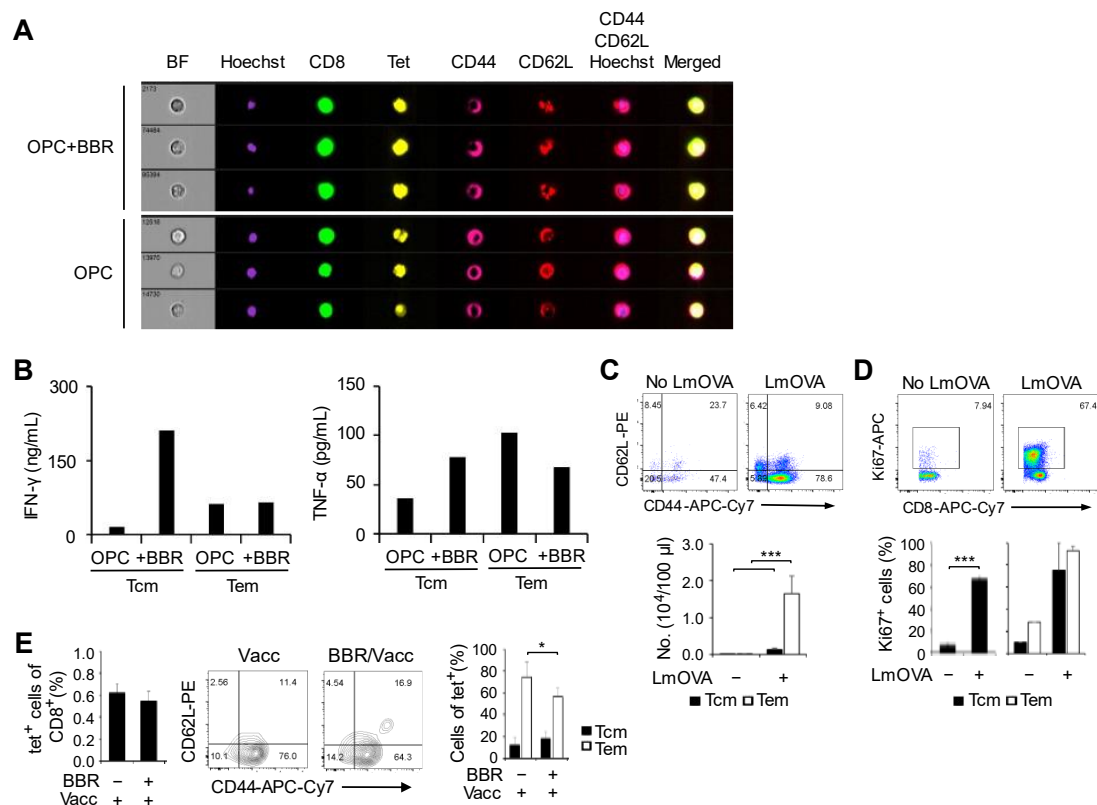


Figure S2 BBR promoted CD8⁺ Tcm differentiate. Cells were cultured, sorted and immunized as described above. (A) Cell morphology was detected by ImageStream Mark II. (B) Production of intracellular IFN- γ and TNF- α . (C) percentage Ki-67⁺CD8⁺ T cells (D) in the recipient's blood 7 days after boost. (E) Percentage of antigen-specific cells and Tcm/Tem. Results are representative of two independent experiments ($n = 3-5$ per group). Data are shown as mean \pm SD; * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$